Development of Chlorine Dioxide Releasing Film and Its Application in Decontaminating Fresh Produce

Soumi Ray, Tony Jin, Xuetong Fan, Linshu Liu, and Kit L. Yam

Abstract: A feasibility study was conducted to develop chlorine dioxide (ClO_2)-releasing packaging films for decontaminating fresh produce. Sodium chlorite and citric acid powder were incorporated into polylactic acid (PLA) polymer. Films made with different amounts of PLA (100 and 300 mg), percentages of reactant (5% to 60%), and ratios of sodium chlorite to citric acid (1:2 or 2:1) were prepared using a solvent casting method. The release of ClO_2 from the resultant films was activated by moisture. Increase of reactants in the films produced more ClO_2 while higher PLA content in the films resulted in less release of ClO_2. The ratio of sodium chlorite to citric acid and activation temperature (22 °C compared with 10 °C) did not affect the ClO_2 release from the films. Antimicrobial efficacy of ClO_2 released from the films was evaluated using grape tomato as a model food. The results indicate that the films were activated by moisture from tomatoes in the package and the released ClO_2 reduced *Salmonella* spp. and *Escherichia coli* O157:H7 inoculated on the tomatoes to undetectable levels (<5 colony forming units (CFU)/tomato), achieving more than 3 log reduction. The film-treated tomatoes did not show significant changes in color and texture as compared to controls during storage at 10 °C for 21 d. This study demonstrated the technical feasibility for development of gaseous ClO_2-releasing packaging system to enhance microbial safety and extend shelf life of fresh produce.

Keywords: antimicrobial packaging, chlorine dioxide, decontamination, fresh produce, moisture activation, pathogens, poly-lactic acid film, quality

Introduction

There has been an increasing demand for fresh and fresh-cut fruits and vegetables due to their health benefits (Beuchat 1996; Mahmoud and others 2007). But the increased consumption also results in an increase number of outbreaks associated with these products (Mahmoud and Linton 2008). Fresh produce has known to be a major vehicle of human pathogens such as *Escherichia coli* O157:H7, *Salmonella spp.*, and *Listeria monocytogenes* (Wilson and others 1991).

Washing with plain water is a common practice to reduce the initial microbial load of fresh produce (Keskinen and Annous 2009), but it alone is sometimes not sufficient to achieve microbial reduction below the desirable safe level. Hence various sanitizers are added to the wash water to kill surface microorganisms (Singh and others 2002). Chlorine and chlorine-based chemicals such as sodium and calcium hypochlorite solutions are generally used for dipping and washing of fresh produce (Wilson and others 1991). However, washing fresh produce with chlorine can reduce only 1 to 2 log colony forming units (CFU) of common bacterial human pathogens (Sweetin and others 1996). Chlorine also reacts with organic materials and produces toxic compounds such as trihalomethanes (THMs) and chloramines (Beuchat 1998; Chang and others 2002; Allende and others 2004; Mahmoud and others 2007). These limitations indicate that the industry needs a more effective sanitizing system to ensure the microbial safety of fresh and fresh-cut produce.

Chlorine dioxide (ClO_2) is an oxidizing agent with strong antimicrobial properties. It is effective over a wide range of pH (3 to 8) and has strong biocidal activity against a broad spectrum of microorganisms including bacteria, fungi, yeast, and mold. It is 3.5 times more powerful than chlorine or chlorinated water (Benarde and others 1965). Either gaseous or aqueous ClO_2 can be used for disinfecting fresh fruits and vegetables (Park and others 2008). A study showed that more than 5 log reduction of *E. coli* O157:H7 on apple skin was achieved when treated with 3.3 mg/L ClO_2 for 20 min or 7.2 mg/L ClO_2 for 10 min (Du and others 2003). Another study showed that 4.3 to 4.7 log reduction of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* was achieved in strawberries when treated with 5.5 mg/L ClO_2 gas for 10 min (Mahmoud and others 2007). Moreover, its effectiveness is not lessened by presence of soil and/or other organic matter and it does not form carcinogenic compounds like chloroamines and THMs because of its inability to react with ammonia, which is a common byproduct of chlorine or chlorinated water treatment (Bellar and others 1974; Woodworth and Jeng 1990).

Yet the current process of washing fresh produce with aqueous ClO_2 has several disadvantages. First, ClO_2 is inconvenient and expensive to use due to its instability and explosive nature. Thus it is usually produced onsite to avoid safety hazards associated with transportation. Second, due to the resistance of surface tension, aqueous ClO_2 cannot reach inaccessible areas such as stomata and crevices where microbes may be attached. Third, once the washing

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process is completed, ClO_2 is no longer available to combat the surviving microbes. To overcome these disadvantages, it is desirable to develop a packaging system that can generate ClO_2 to eliminate its cost of production in the manufacture plant. Instead of washing with aqueous ClO_2, gaseous ClO_2 is released from the package to the fresh produce. Gaseous ClO_2 has the advantage that it can reach areas inaccessible to aqueous ClO_2. Ideally, this is a controlled release packaging system (LaCoste and others 2005; Chen and others 2012; Zhu and others 2012) in which gaseous ClO_2 is released in a controlled manner to provide prolong protection against surviving microbes.

Several studies investigated the killing effect of ClO_2, prepared using acidic solution of sodium chlorite or sodium chlorate as precursor of ClO_2 (Tang and Gordon 1984; Tenney and others 1990; Deshwal and Lee 2004; Deshwal and others 2004; Jin and others 2009; Aday and Caner 2011). To the best of our knowledge, there is no literature report on the development of a ClO_2 gas-releasing packaging system that can be activated by moisture from fresh produce.

The objective of this study was to investigate the feasibility of developing an antimicrobial packaging film that can self-generate ClO_2 via a chemical reaction activated by moisture from fresh produce and then release the ClO_2 gas to inhibit the microbial growth of the product. To achieve this objective, the research tasks were conducted to (1) develop a ClO_2-releasing packaging system in the form of film by incorporating sodium chlorite and citric acid powder in polylactic acid (PLA) polymer, (2) determine the release of ClO_2 from the films activated by moisture, and (3) evaluate antimicrobial activity of the film against foodborne pathogens and potential application in fresh produce using grape tomato as a model food.

Materials and Methods

Materials

Technical grade sodium chlorite was obtained from Ricca Chemical Co. (Arlington, Tex., U.S.A.) and sulfuric acid was obtained from Fisher Chemicals (N.J., U.S.A.). Anhydrous citric acid and methylene chloride were obtained from Acros Organics (N.J., U.S.A.). PLA (4060D) was obtained from Natureworks (Minnetonka, Minn., U.S.A.) and Rhodamin B was purchased from Alfa Aesar (Lancs, U.K.).

Preparation of ClO_2 releasing films

Films were prepared by a solvent casting method. PLA resin was dissolved in 10 mL methylene chloride, and sodium chlorite was added to the PLA solution followed by citric acid with continuous stirring to facilitate homogeneous mixing. The mixture was then casted on Teflon Petri dish (11 cm in dia) and dried in a closed chamber with continuous dry air flow at ambient temperature (22 ± 1 °C). Films were peeled off from the Petri dishes and stored in ziplock bags to prevent absorption of moisture from environment. Nine types of films were prepared with different PLA percentage (PLA in solution), reactant percentage (sodium chlorite and citric acid in PLA), and salt to acid ratio (sodium chlorite to acetic acid in reactants) as shown in Table 1.

Quantification of ClO_2 gas released from films

Preparation and standardization of ClO_2 stock solution. ClO_2 stock solution was prepared by dissolving 10 g sodium chlorite in 500 mL water in a gas generating glass bottle. Sulfuric acid (20% (v/v)) was added intermittently through a separating funnel fitted in the gas generating bottle. ClO_2 gas produced was passed through a saturated solution of sodium chlorite to remove any acid vapor mixed with ClO_2 gas. Finally stock solution was prepared by bubbling the ClO_2 gas through distilled water. This solution was collected in a dark glass bottle and stored at 4 °C. Stock solution was standardized by measuring absorbance at 360 nm. Dilute standard solutions were prepared from the stock solution and a standard curve was generated using Rhodamine B (Xin and Jinyu 1995).

Table 1–Compositions of chlorine dioxide releasing films[a].

Film	PLA (mg)	NaClO2 (mg)	Citric acid (mg)	Reactant (%) (wt/wt)	Salt–acid ratio
1A	100	13.5	6.5	20	2:1
1B	100	6.5	13.5	20	1:2
1C	100	40	20	60	2:1
3A	300	40	20	20	2:1
3B	300	20	40	20	1:2
3C	300	10	5	5	2:1
3D	300	60	30	30	2:1
3E	300	30	60	30	1:2
3F	300	50	25	25	2:1

[a]Ten milliliters of methylene chloride was used for each film.

Determination of quantity of ClO_2 released from film activated by environmental moisture. The film was stuck inside wall of a tightly closed 250 mL mason jar with 0.5 mL water on the bottom to activate the chemical reaction. Headspace (2 mL) was withdrawn at predetermined intervals with a gas tight syringe through the septum fitted on the lid and dissolved in DI water in 4.5 mL amber glass vial fitted with Teflon screw top caps (Pepich and others 2007). The concentration of ClO_2 in the vial was determined from the standard curve and concentration of ClO_2 in headspace was calculated. Average ClO_2 concentration of 3 films was used for all calculations. The release study was conducted at ambient temperature (22 ± 1 °C) and 10 °C.

Determination of quantity of ClO_2 released from a film activated by fresh produce. The release of ClO_2 from the film inside in a clam shell container (24 oz) with tomatoes was determined using ClO_2 detecting tubes (Gastek, model 23M). ClO_2 was measured for each treatment on day 2, 7, 14, and 21 before opening the containers for quality evaluations. Fifty milliliters of package headspace was withdrawn with the pump and passed through the tube fitted with pump and held for 1 min. Concentration of ClO_2 obtained from tube reading was multiplied by 2 to normalize for 100 mL air.

Antimicrobial activity of films

Inoculum preparation. *E. coli* O157:H7 ATCC 43894 and a cocktail of *Salmonella* Panama 19454, *Salmonella* Poona 953, and *Salmonella* Stanley H0558 were used in this study. All strains were taken from the culture collection of the U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center and were maintained in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md., U.S.A.) at 2 °C and transferred bimonthly. Each fresh culture (10 mL) was grown overnight (18 h) in TSB at 37 °C for use in experiments. The cell concentration of inoculum was approximately 10^9 CFU/mL, as determined by serial dilution in 0.1% peptone water, and 100-μL aliquots of various dilutions were spread plated onto tryptic soy agar (TSA; Difco, Becton Dickinson) and then incubated for 24 h at 37 °C.

Sample preparation and film treatment. Grape tomatoes were bought from a local supermarket, were washed and rinsed

with 70% ethanol to eliminate any possible background microorganisms. Samples were inoculated under biohood by spot-inoculation with 40 μL inoculum. Inoculated samples were put in a clam shell box (24 oz) with the film (11 cm^2) stuck on the lid of the box, then the box was sealed and stored for 24 h at 22 °C and 10 °C before microbiological analysis. Inoculated samples without films in the boxes served as controls.

Microbiological analysis. Two tomatoes were randomly selected from a box and placed in an individual Whirl-Pak bag containing 10 mL of D/E Neutralizing Broth (BD), and hand-massaged for 1 min. Each homogenate was serially diluted in 0.1% peptone water (pH 6.9) (0.1 mL in duplicate) and surface plated on CT-SMAC for *E. coli* O157:H7 or TSA with 0.1% sodium pyruvate and 100 ppm nalidixic acid (TSAPN) for *Salmonella*. All plates were incubated at 37 °C for 24 h before counting CFU.

Quality analysis

Sample preparation. A single layer of grape tomatoes (175 to 180 g) was placed in each clam shell box. ClO_2 releasing film was taped on the inner lid of clam shell. Samples were stored at 10 °C to mimic commercial practice. The tomato samples in a box without any film served as controls. Treated samples and controls were withdrawn after 2, 7, 14, and 21 d to measure color and texture. ClO_2 concentrations inside the package were also

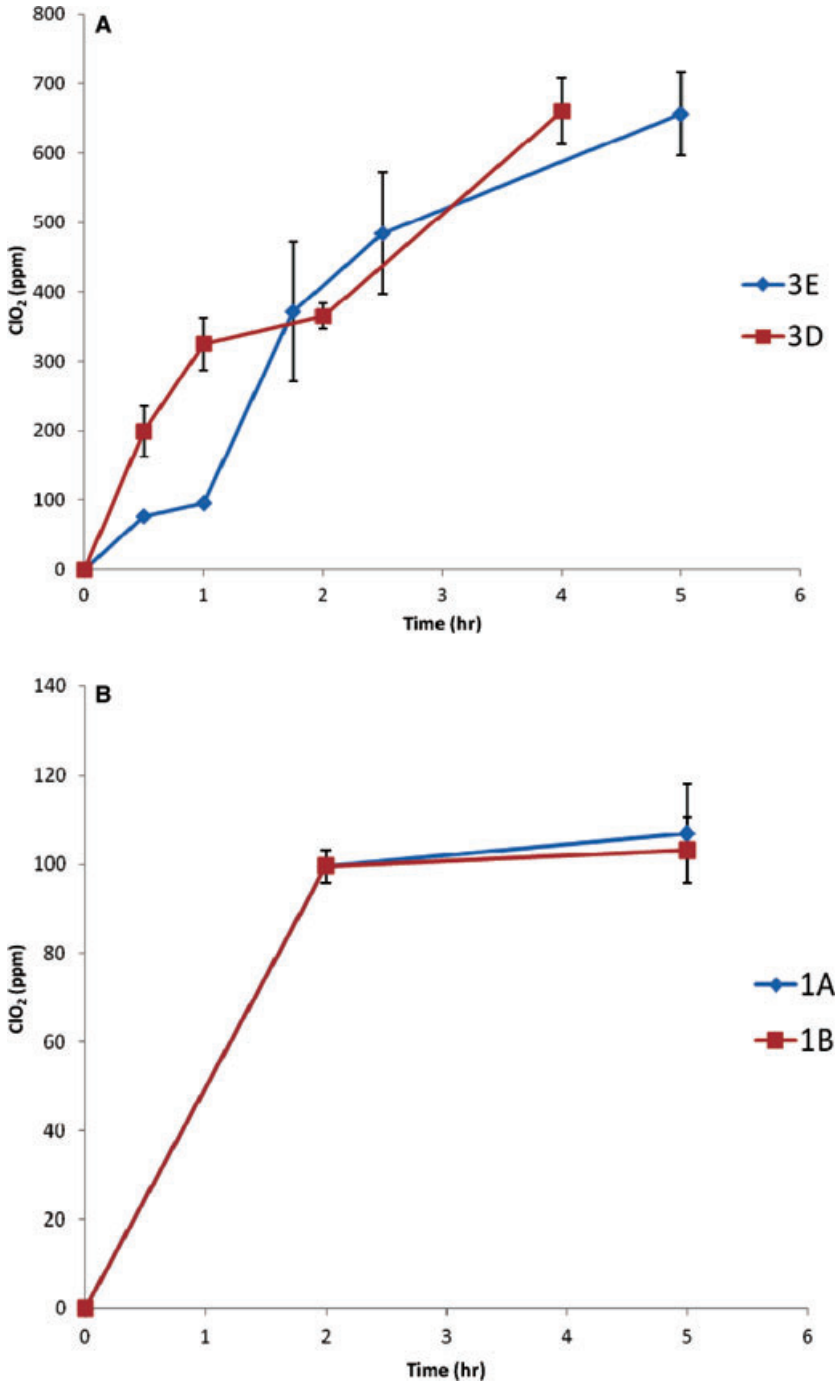


Figure 1–Effect of salt–acid ratio on release of chlorine dioxide from film: (A) films made with 300 mg PLA; (B) films made with 100 mg PLA. 3D: 300 mg PLA, 30% reactants, 2:1 salt:acid; 3E: 300 mg PLA, 30% reactants, 1:2 salt:acid; 1A: 100 mg PLA, 20% reactants, 2:1 salt:acid; 1B: 100 mg PLA, 20% reactants, 1:2 salt:acid.

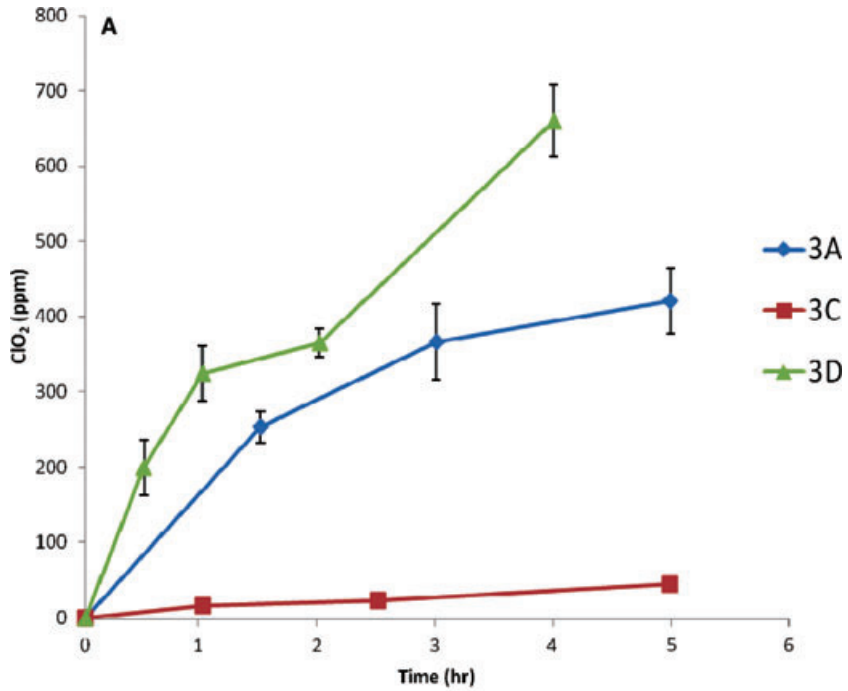
measured prior to quality analysis of the fruits as mentioned. The whole experiment was done in triplicates.

Color measurement. Surface color of tomato was evaluated using Hunter UltraScan® VIS colorimeter (Hunter Associates Lab, Reston, Va., U.S.A.) with 1.3 cm measuring aperture. Lightness (L^*), redness (a^*), and yellowness (b^*) were recorded and Hue (atan (b^*/a^*) × 57.3) and chroma ($\sqrt{(a^{*2} + b^{*2})}$) values were calculated from a^* and b^*.

Texture measurement. Firmness of treated and untreated tomatoes was evaluated with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). A 3 mm dia probe was used to penetrate the fruit to 10 mm depth at 10 mm/s. Texture was reported in terms of firmness or softness and expressed as force (g) required for puncturing tomatoes.

Statistical analysis

All experiments were replicated 2 or 3 times. Data were analyzed using analysis of variance (ANOVA) with SAS version 9.2 software (SAS Inst., Cary, N.C., U.S.A.). Bonferroni least significant difference (LSD) test was used to determine the significant differences of mean values. Significance was defined at $P \leq 0.05$. Data points were expressed as the mean ± standard deviation.



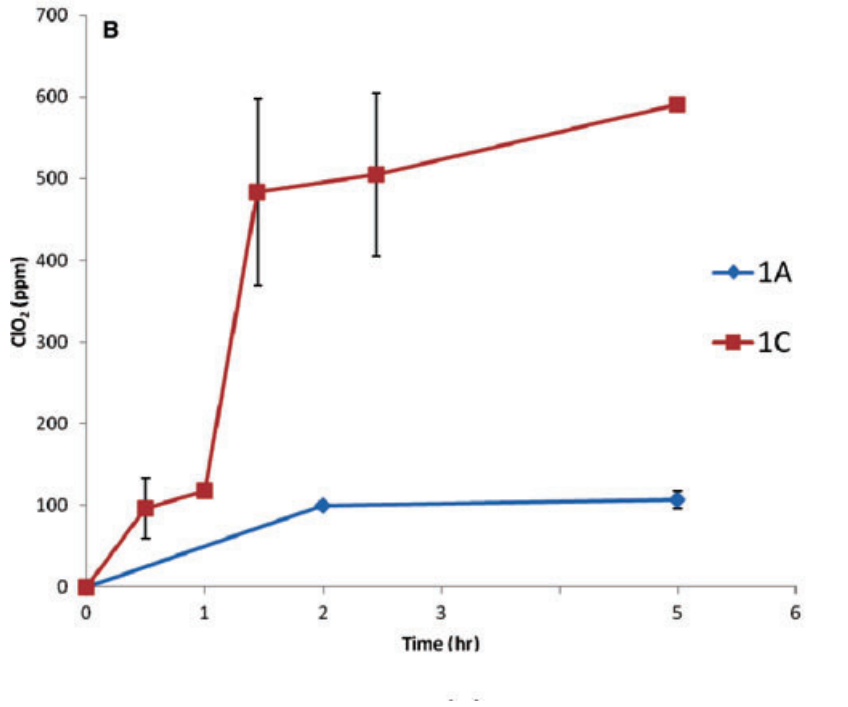


Figure 2–Effect of reactant percentage on release of chlorine dioxide from film: (A) films made with 300 mg PLA; (B) films with 100 mg PLA.
3A: 300 mg PLA, 20% reactants, 2:1 salt:acid;
3C: 300 mg PLA, 5% reactants, 2:1 salt:acid;
3D: 300 mg PLA, 30% reactants, 2:1 salt:acid;
1A: 100 mg PLA, 20% reactants, 2:1 salt:acid;
1C: 100 mg PLA, 60% reactants, 2:1 salt:acid.

Results and Discussion

ClO_2 released from films

The effects of salt–acid ratio, reactant percentage, film thickness, and reaction temperature on ClO_2 release profile were investigated. The release of ClO_2 from films was monitored for up to 12 h. In most cases, maximum release of ClO_2 was achieved at or before 5 h. Therefore, results up to 5 h were reported.

Effect of salt–acid ratio. ClO_2 released from films with same reactant percentage but at different salt–acid ratio was determined. Films made with 300 mg PLA and 30% reactants at salt–acid ratio of 2:1 (3D) showed a different release profile from that at salt–acid ratio of 1:2 (3E) during 5 h exposure to moisture (Figure 1A). However, the maximum ClO_2 released from both films was not significantly different from each other, which were achieved at 4 and 5 h, respectively. A similar trend was observed for the films made with 100 mg PLA and 20% reactants (Figure 1B). The results match those obtained in our preliminary study when the reactants were directly mixed together without incorporating into PLA. However, the ClO_2 released from films was less than that from direct mixture when the same amount of reactants was used (data not shown). This may be because when salt and acid molecules

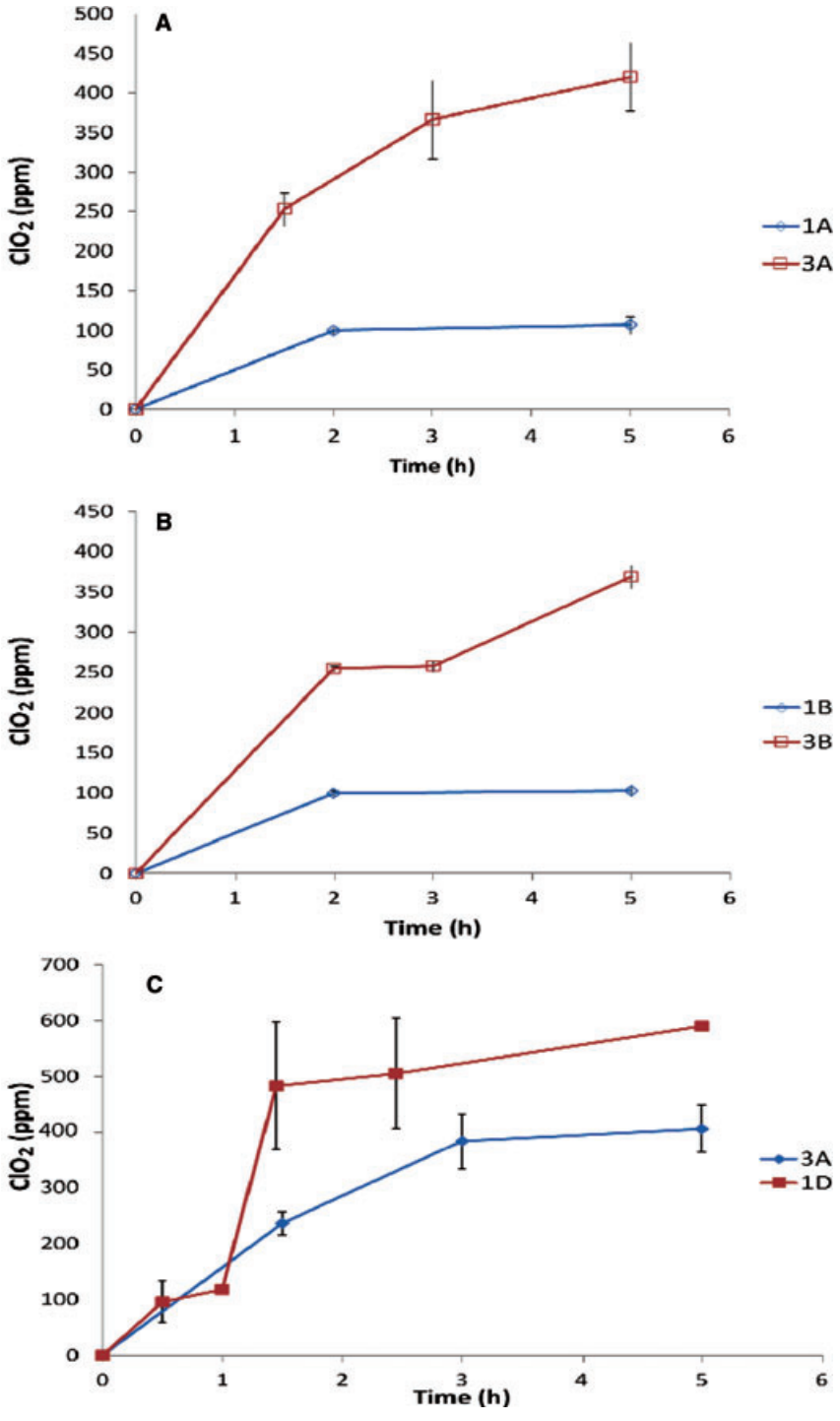


Figure 3–Effect of film thickness on release of chlorine dioxide from film: (A) films at salt–acid ratio of 2:1; (B) films at salt–acid ratio of 1:2; (C) films with same amount of reactants.
1A: 100 mg PLA, 20% reactants, 2:1 salt:acid;
3A: 300 mg PLA, 20% reactants, 2:1 salt:acid;
1B: 100 mg PLA, 20% reactants, 1:2 salt:acid;
3B: 300 mg PLA, 20% reactants, 1:2 salt:acid;
1C: 100 mg PLA, 60% reactants, 2:1 salt:acid.

are distributed within PLA matrix, there is some additional barrier between these molecules and not all of them are in direct contact, which is one of the key factors for production of ClO_2. Ratio of salt to acid did not affect the quantity of ClO_2 released from films, suggesting salt to acid ratio is not a critical parameter for controlling ClO_2 release.

Effect of reactant percentage. Reactant percentage is defined as the percentage of total reactants (acid and salt) in PLA. As expected, an increase of reactants in PLA films significantly increased the concentration of ClO_2 released from the films during 5 h (Figure 2). ClO_2 released from the film with highest reactant (30%) was 670 ppm at 4 h, while films with 20% and 5% reactants generated 420 ppm and 30 ppm ClO_2 at 5 h, respectively (Figure 2A). Similar results were observed with 100 mg PLA films, the film with 60% reactants reached 600 ppm at 5 h while that with 20% reactants only had 100 ppm (Figure 2B). These data indicate that reactant percentage is a critical parameter for controlling the release of ClO_2 from films.

Effect of film thickness. The thickness of PLA film containing 100 mg PLA was 0.11 mm and that of 300 mg PLA was 0.13 mm. ClO_2 released from film made with 300 mg PLA film (420 ppm) was greater than that released from film made with 100 mg PLA film (110 ppm) at 5 h, both films containing same reactant percentage (Figure 3A). Similar results were observed in the films at 1:2 acid–salt ratio (Figure 3B). Because reactant percentage was related to PLA content and increase in PLA content (from 100 to 300 mg) also increased the concentration of reactants in the films, the effect of thickness was actually positively associated with the concentration of reactants in the film. Therefore, another test was conducted to test the real effect of film thickness on ClO_2 release. Both films (300 mg PLA and 100 mg PLA) containing same amount of reactants but not same reactant percentage (1C compared with 3A) were used in this test. The increase of PLA in the film resulted in an increase of film thickness and consequently reduced the release of ClO_2 (Figure 3C). These results suggested that film thickness can be a controlling factor for release of ClO_2 as showed in Figure 3C, where release of ClO_2 reduces with increase in film thickness, keeping the amount of reactant same in both the films. However, ClO_2 release increases when both film thickness and reactant concentration in the film increases (Figure 3A and B). As production of ClO_2 is a relative humidity-dependent reaction and PLA is a hydrophobic polymer (Buddy 1995; Burg and others 1999; Rasal and others 2009), sufficient moisture could not reach inside the polymer matrix (Yew and others 2005). Therefore, ClO_2 was produced only from sodium chlorite and citric acid present on the film surface. For relative humidity-dependent reactions, hydrophilic polymers would be a better choice to achieve controlled release as moisture cannot penetrate inside hydrophobic polymers; reactants present only on the surface of the hydrophobic films would affect the release and molecules entrapped within the polymer matrix would not participate in the reaction or diffuse toward the surface. Another approach could be modification of hydrophobic polymers like PLA with various hydrophilic materials to have a composite with increased moisture absorption capacity (Yew and others 2005; Qin and others 2010).

Table 2– Survival of *Salmonella* and *E.coli* O157:H7 on grape tomato.

Microorganism	Temperature	Treatment	Bacterial population (log CFU/tomato)
Salmonella spp.	22 °C	Control	4.26 ± 0.23
		3C	1.08 ± 0.26
		3D, 3E, 3F, 1C	ND[a]
	10 °C	Control	5.17 ± 0.35
		3C, 3D, 3E, 3F, 1C	ND
E. coli O157:H7	22 °C	Control	3.61 ± 0.01
		3C, 3D, 3E, 3F, 1C	ND

[a]ND, not detectable (<5 CFU/tomato).

Effect of temperature. One film with highest release rate (3E) was selected for a comparison test at 22 °C and 10 °C. Figure 4 shows that the film at both temperatures had similar release patterns. However, the film at room temperature released slightly more ClO_2 than that at 10 °C during 1 to 2.5 h while the release rate of film at 10 °C was slightly higher than that at room temperature during 2.5 to 5 h. This suggests that activating

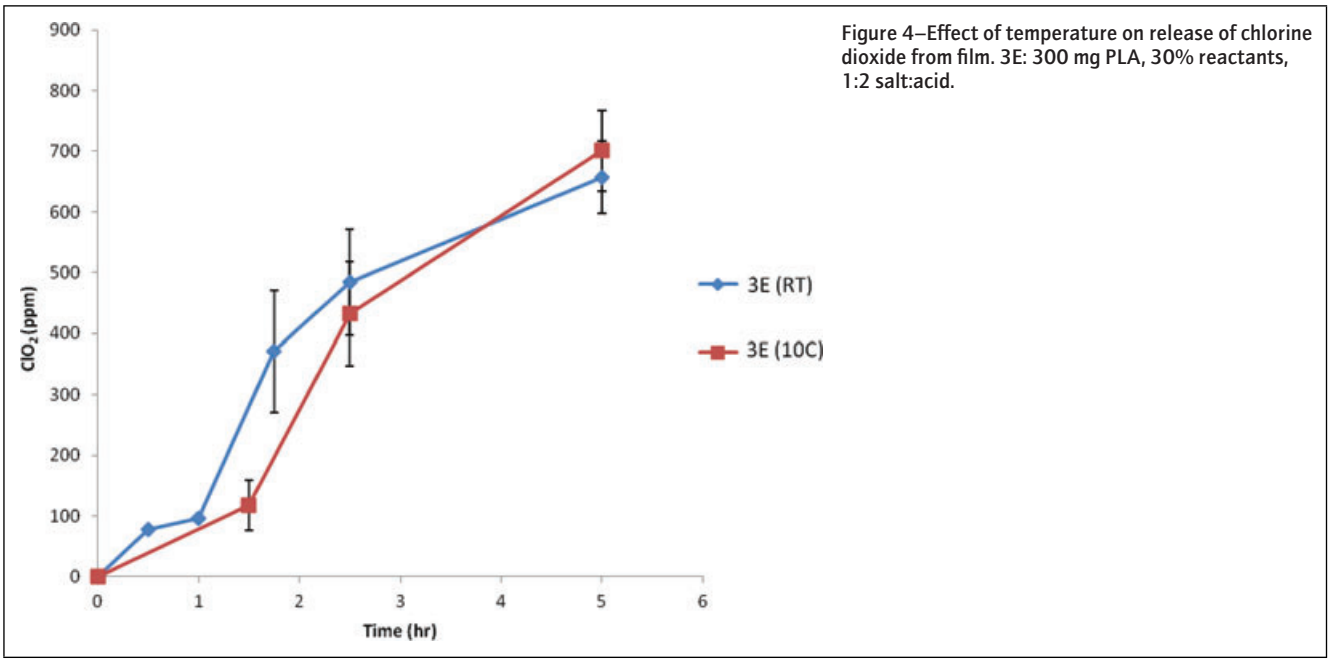


Figure 4–Effect of temperature on release of chlorine dioxide from film. 3E: 300 mg PLA, 30% reactants, 1:2 salt:acid.

Table 3–Changes in color of tomato skin during storage at 10 °C.

Parameter	Treatment	Storage time (d) 0	2	7	14	21
L*	Control	35.58 ± 1.89	33.14 ± 1.24^{a}	33.72 ± 1.27^{ab}	33.76 ± 1.34^{a}	33.20 ± 1.25^{a}
	3C	35.58 ± 1.89	33.57 ± 1.56^{a}	33.59 ± 1.40^{ab}	33.32 ± 1.06^{a}	32.86 ± 1.20^{ab}
	3D	35.58 ± 1.89	33.57 ± 1.73^{a}	33.04 ± 1.08^{b}	33.42 ± 1.08^{a}	32.37 ± 1.19^{b}
	3E	35.58 ± 1.89	33.22 ± 1.35^{a}	33.68 ± 1.32^{ab}	33.84 ± 1.41^{a}	33.19 ± 1.57^{a}
	3F	35.58 ± 1.89	33.74 ± 1.70^{a}	33.81 ± 1.74^{a}	33.42 ± 1.31^{a}	33.02 ± 1.47^{ab}
	1C	35.58 ± 1.89	33.16 ± 1.32^{a}	33.65 ± 1.33^{ab}	33.26 ± 1.36^{a}	33.12 ± 1.10^{ab}
Chroma value	Control	31.21 ± 2.9	29.09 ± 2.84^{ab}	29.30 ± 3.35^{a}	29.64 ± 3.11^{a}	29.61 ± 3.37^{a}
	3C	31.21 ± 2.9	29.15 ± 2.86^{ab}	28.88 ± 2.54^{a}	28.46 ± 2.71^{a}	29.31 ± 3.47^{a}
	3D	31.21 ± 2.9	28.14 ± 3.48^{b}	28.64 ± 2.26^{a}	29.39 ± 2.34^{a}	27.54 ± 2.34^{a}
	3E	31.21 ± 2.9	29.43 ± 2.86^{ab}	29.44 ± 2.64^{a}	29.82 ± 2.89^{a}	28.72 ± 3.09^{a}
	3F	31.21 ± 2.9	30.47 ± 2.9^{a}	29.37 ± 2.46^{a}	28.70 ± 2.99^{a}	28.78 ± 3.64^{a}
	1C	31.21 ± 2.9	28.71 ± 2.75^{b}	29.59 ± 2.81^{a}	29.35 ± 2.08^{a}	29.25 ± 2.82^{a}
Hue value	Control	41.39 ± 2.84	39.63 ± 2.61^{a}	39.71 ± 2.69^{a}	39.77 ± 2.30^{a}	39.09 ± 2.11^{ab}
	3C	41.39 ± 2.84	40.07 ± 2.54^{a}	39.51 ± 2.06^{a}	39.20 ± 2.68^{a}	38.89 ± 2.30^{b}
	3D	41.39 ± 2.84	41.24 ± 4.55^{a}	38.80 ± 2.34^{a}	39.21 ± 2.37^{a}	39.74 ± 2^{ab}
	3E	41.39 ± 2.84	40.09 ± 2.69^{a}	39.88 ± 2.39^{a}	40.27 ± 2.57^{a}	40.37 ± 3.18^{a}
	3F	41.39 ± 2.84	40.60 ± 2.94^{a}	39.62 ± 2.76^{a}	39.71 ± 2.18^{a}	40.05 ± 2.64^{ab}
	1C	41.39 ± 2.84	40.68 ± 2.51^{a}	38.8 ± 2.34^{a}	39.56 ± 3.04^{a}	40.05 ± 2.92^{ab}

Data are means ± standard deviation. Means in the same column with different letters are significantly different ($P < 0.05$).

temperature in general did not affect the release dramatically and therefore these films are suitable for use at 22 °C or 10 °C.

ClO_2 concentration inside package. Concentrations of ClO_2 gas inside each package containing tomatoes were measured at 2, 7, 14, and 21 d using ClO_2 gas detecting tubes. Similar to film tests in glass jars, packages with films at higher reactant percentage had higher concentration of ClO_2 when measured during a same period of time. However, ClO_2 depleted in packages with all the films over time and reached below detection limit after 7 d of storage (data not shown). This result is expected and could be combination of several factors, such as, absorption of the gas by fruit, loss of gas through seals and leaks of the package, and decomposition of ClO_2 gas molecules over time as it can participate into a series of reaction and can degrade to chlorite and chlorate, which cannot be detected by ClO_2 detecting tubes used in this research (Kim and others 1999).

Microbial activity of ClO_2 from films

Preliminary screening work was done to determine antimicrobial activities of 9 types of films initially developed, and 5 films of those 9 films, which showed more antimicrobial activity against the pathogens in microbial media, were selected for further study in tomatoes. Table 2 shows the survival of *Salmonella* spp. and *E. coli* O157:H7 after 24 h treatments with ClO_2 releasing films. Control samples at 22 °C had 4.26 and 3.61 log CFU/tomato of *Salmonella* and *E. coli* O157:H7, respectively. No *Salmonella* or *E. coli* cells were detected (<5 CFU/tomato) in any treatment except for the treatment with the film containing 5% reactant and 3% PLA (3C) for *Salmonella*. Additional test for *Salmonella* at 10 °C showed that all film treatments reduced the pathogen to undetectable levels (Table 2). The least antimicrobial activity of 3C film was expected since the film releases least ClO_2 gas during the release study (Figure 2A). In the present study, the ClO_2 releasing films achieved minimum 3 log reduction of pathogens on tomatoes. More microbial reduction could be achieved if tomato surface contained higher initial bacterial cells, which needs to be further investigated. Antimicrobial effectiveness of the films in this study is comparable with many ClO_2 gas treatments that have been studied so far. Mahmoud and others (2007), Richards and Beuchat (2004), and Sy and others (2005) reported 4.7 log reduction/strawberry and 4.3 log reduction /tomato when treated with 5 mg/L ClO_2 for 10 min and 4.1 mg/L for 25 min, respectively. Another study showed that approximately 4.87 log reduction/cm^2 of tomato was achieved with 10 mg/L ClO_2 for 180 s (Trinetta and others 2010).

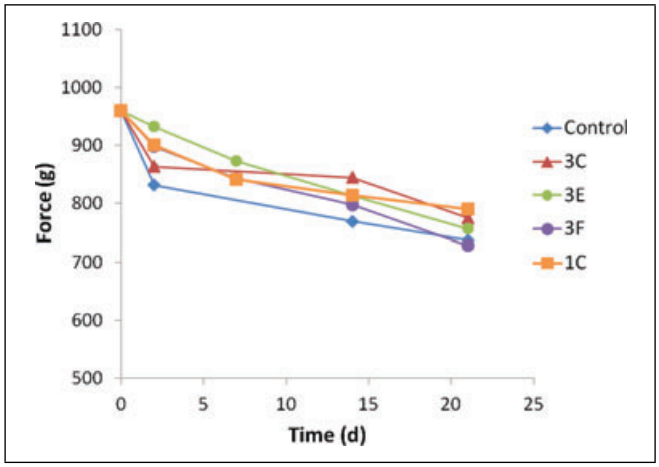


Figure 5–Effect of chlorine dioxide film treatments on texture of grape tomatoes.

Quality of tomatoes affected by film treatments

Color. Changes in tomato color were monitored during storage at 10 °C for 21 d. L*, Chroma, and Hue values were reported in Table 3. During storage, L*, Chroma, and Hue values decreased slightly for all samples including controls, however, statistical analysis shows that there was no significant difference ($P > 0.05$) in color change among all the samples, indicating that none of the films had a deleterious effect on tomato color during 21 d of storage at 10 °C. Other studies have shown that treatment with ClO_2 gas and solution did not significantly affect the color of roman tomatoes (Trinetta and others 2010) and strawberry (Kim and others 2010), which agree with our findings. Other researchers also reported that treatment with ClO_2 gas did not affect the external color and visual appearance of strawberry and whole cantaloupe (Mahmoud and others 2007, 2008). Therefore, the films in these

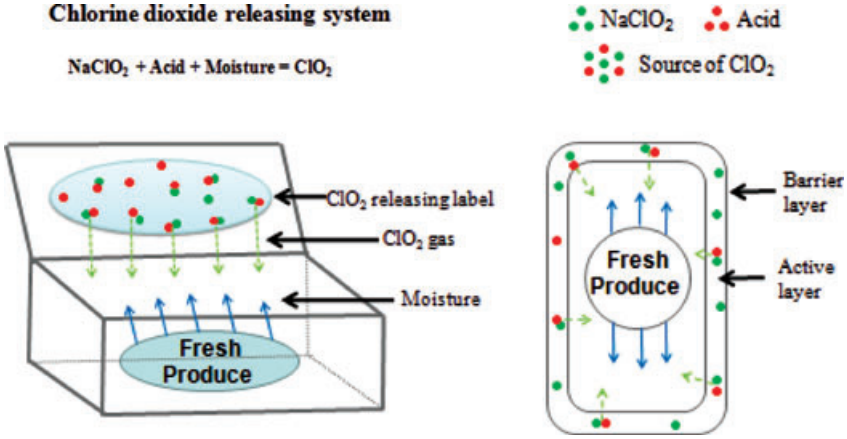


Figure 6–Concept and mechanism of chlorine dioxide releasing packaging system.

ranges of reactant percentage (5% to 30%) used in our study are suitable for grape tomatoes.

Texture. Changes in texture of tomatoes during storage at 4 °C were observed for all the samples including controls (Figure 5). However, statistical analysis showed that no significant difference ($P > 0.05$) in texture changes among the film treatments. Apart from firmness, researchers have studied whether ClO_2 treatment causes any shrinkage or wrinkling of the fruit skin. Trinetta and others (2010) reported that treatment with 10 mg/L ClO_2 gas (36000 ppm) for 180 s resulted in 4.87 log reduction of *Salmonella* but caused wrinkle on the tomato skin (Trinetta and others 2010). However, in our study films with highest reactant concentration (3D and 3E) had almost equal (at 22 °C) or even better (at 10 °C) antimicrobial effect but did not cause wrinkle or any other visually perceivable changes in the sample. This may indicate that a longer time exposure at lower concentration has a better effect than high concentration—short time exposure. Possible reason could be as film releases ClO_2 slowly over a long period of time, samples get enough time to adapt to slowly increasing ClO_2 environment instead of exposing them to a very high concentration at once.

Sensory. An informal sensory study was conducted and could not observe any off-flavor and off-odor in fruit. However this needs to be validated with a larger group.

The number of documented outbreaks of foodborne diseases associated with the consumption of raw fruits and vegetables has increased in recent years. The possibility of human pathogen contamination on foods after sanitization or thermal processing could result from package machines; package materials; poor hygiene by packinghouse workers or supermarket employees; or cross-contamination in the supermarket during unpacking, display, in-store fresh-cut processing, or handling. Since ready-to-eat (RTE) foods such fresh fruits or other deli foods are consumed without prior cooking, controlling the growth of the pathogens that may contaminate RTE foods is particularly important for ensuring the microbial safety of those products. Therefore, there is an urgent need for intervention methods used as nonthermal in-package pasteurization for RTE foods.

The central idea behind the project was to develop an in-package pasteurization system that would improve food safety without negatively impacting on food quality as shown in Figure 6. ClO_2 is a well-established antimicrobial agent currently used as gas or in solution for disinfecting and sanitizing fresh produce. The ClO_2-releasing packaging provides advantages over the current ClO_2 washing practice by avoiding possible cross-contamination or recontamination of foods after packaging and before consumption. An important requirement for making ClO_2-releasing films was the need to prevent environmental moisture during film making and storage before the films are used for packaging food. In this study, a solvent casting method and dry air were used. In addition, a weak organic acid (citric acid) was used to further reduce the sensitivity of reactants to possible environment moisture during film making and storage. For large-scale production, it is a challenge to make such films using extrusion technology. However, it should be achievable if a moisture-free work environment could be used.

The reactants could be incorporated to other polymers. However, PLA polymer is biosourced and biodegradable, the use of PLA in antimicrobial packaging could provide additional environmental benefits as compared to other petroleum polymers. The results in this study show effectiveness of the films in inactivating pathogens on grape tomatoes without impacting the quality. The same antimicrobial packaging could also be applied for other fresh produce that can generate moisture inside of a package. By adjusting PLA percentage and reactant percentage in a film, different quantity of ClO_2 released from the film could be achieved for different kind of fresh produce. The application could also be extended to non-moisture-generated foods, when a drop of water is injected into the package to activate ClO_2 release.

This is a 1st report and a preliminary study, which provides a starting point to develop an in-package antimicrobial system. Further research is needed to translate this concept into practice.

Conclusions

Sodium chlorite and citric acid can be incorporated into PLA films. The amount of moisture generated by respiration of grape tomato was sufficient to activate the chemical reaction and facilitate release of ClO_2 gas. The ClO_2 released from the films inside the package effectively inactivated *E. coli* O157:H7 and *Salmonella* spp. on grape tomatoes, achieving more than 3 log reduction of both pathogens and yet not affecting texture, color, and visual appearance of grape tomatoes during storage for 21 d at 10 °C. This study demonstrated the technical feasibility to develop a gaseous ClO_2-releasing packaging system and its potential application for decontaminating grape tomato, and perhaps can be extended to other fresh produce.

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